COMPARATIVE STUDY OF ANTIBIOTICS, TOOTHPASTE AND PLANT EXTRACTS AGAINST BACTERIAL PATHOGENS ISOLATED FROM HUMAN GUMS.

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ABSTRACT

This study was conducted to find out the effectiveness of some antibiotics, toothpastes, and medicinal plant extracts against the bacterial species isolated from human gums. A total of 125 samples were collected. The male and female patients were with the age from 15-60 years. A total of 27 species were isolated and identified. Among those bacterial species, Streptococcus mutans was most prevalent 29.62%, followed by Lactobacillus acidophilus 22.22%, Streptococcus sobrinus 18.51% and S aureus 14.81% respectively. While Micrococcus spp. 3.7%, Streptococcus sanguinis 3.7%, Actinomyces viscosus 3.7% and Aeromonas were least prevalent. To observe the most effective treatment for gum infections, the isolated strains were subjected to the sensitivity tests against antibiotics, toothpastes and plant extracts. The maximum zone of inhibition formed by Sulfamethoxazole was found against Staph aurus 33mm, while the minimum zone by Amoxicillin was observed against Streptococcus sobrinus (6mm). The maximum zone of inhibition by Toothpaste 1 (Fluoride and salt) was against Micrococcus spp. 34mm. The maximum zone of inhibition by Curcuma longa against S. mutans was 22mm, while the minimum zone by Ajuga bracteosa against S. mutans was 11mm. The data obtained through this study revealed that antibiotics were more effective for the treatment of oral bacterial pathogens as compared to toothpaste and plant extracts with moderate and low activity, respectively. Therefore, it is strongly suggested that use of antibiotics during infection and toothpaste with fluoride and salt contents in daily routine could help in the elimination of bacterial pathogens to maintain oral health.

Keywords: Ajuga bracteosa, Curcuma longa, Toothpaste, Antibiotics

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INTRODUCTION

Microbial investigations have revealed that diverse array (trillions) of microorganisms resides on different parts of the body. One such favorable place for these microbes is the human mouth. (Prasad et al., 2007). The

human oral cavity possesses a microbial population which is very complex and distinctive. The oral microbes have direct relationships with other microorganisms and with the human host in niche-specific associations. The disruption of homeostasis that ensures a balanced microbial composition or dysbacteriosis can contribute to numerous oral infections, notably dental caries and gum diseases (Balachandran et al., 2020).

According to Human oral microbiome database (HOMD), there are 150 genera of microorganisms identified as common causes of oral diseases among them Streptococcus, Haemophilus, Actinomyces, and Prevotella have higher abundance than any other genera. (Gao et al., 2018). More than 700 microbial species have been found to be present inside a human oral cavity by non-culturing technique. Most of the bacteria of the oral cavity are benign and like in other parts of the body; they are in a symbiotic relationship. However, few can cause many gum diseases. Due to changes in home environmental conditions, though, the mucosal surface microbiota shows compositional differences with that present in dental plaque (Zaura et al., 2015).

Among other various identified bacteria species from the oral cavity, Lactobacillus and Streptococcus spp. are the dominant one. The most common opportunistic pathogen among all is Streptococcus mutants (Burton et al., 2011; Gamboa et al., 2004). However, Staphylococcus aureus is a significant pathogen prefer mouth and hands as main reservoirs for propagation and colonize than other several anatomical sites in the human body (Tanomaru et al., 2008).

Streptococcus mutans is the major cause of tooth decay is a Gram-positive, non-spore forming, catalase-negative, non-motile, facultative anaerobic cocci (David et al., 2011). The Mutans streptococci include a group of seven species, of which Streptococcus mutans and Streptococcus sobrinus are the principal species obtained from saliva and plaque of human (Loescheet al., 1986). Prevention of dental caries can be achieved by proper and regular tooth brushing and rinsing with mouth rinses containing antibacterial agent such as chlorhexidine and sodium hypochlorite (Chany et al., 2001).

The antibiotics such as penicillin, amoxicillin, ampicillin, erythromycin tetracycline and chloramphenicol are used dental treatment caries (AI-Haroni et al., 2007). The antibiotics for the prevention of dental caries are not recommended since there is a risk of developing bacterial resistance. The xylitol sugar has inhibited the growth of Streptococcus mutans and not used as an energy source by the cariogenic bacteria, and acid production is reduced (Lynch, H et al., 2003). While dental biofilm cannot be eradicated, it can be minimized and managed through daily oral care and a routine mechanical oral hygiene procedure (Gurenlian et al., 2007). Streptococcus mutans that is the major cause of tooth decay is Gram positive, no spore formation, no catalase, non-motile, facultative anaerobic bacteria (David et al., 2011). The S. Mutans include seven species group, of which Streptococcus mutans and Streptococcus sobrinus are the principal ones obtained from saliva of the patients and plaque of human (Loesche et al., 1986). To prevent dental caries regular tooth brushing and periodic use of mouth washes having antibacterial mediators e.g., chlorhexidine and sodium hypochlorite. (Chany et al.,2001). Antibiotics frequently used to treat dental caries are penicillin, amoxicillin, ampicillin, erythromycin tetracycline and chloramphenicol (AI-Haroni et al., 2007). To prevent dental caries antibiotics are not suggested, as there is risk factor involved to obtain bacterial resistance. The xylitol (substitute sugar) has tendency to inhibit the growth of S. mutans and interestingly do not consumed by the cariogenic bacterial species and also reduce acid production (Lai et al., 2012). While risk of dental biofilm production can never be eliminated, it can only be decreased and maintained by regular oral care along with repetitive mechanical means of oral hygiene practices (Gurenlian et al., 2007). The purpose of this research study was to identify bacteria responsible for gums diseases in the Pakistani population and to analyze their susceptibility against various antibiotics, popular toothpaste brands containing fluoride and plant extracts which were thought to be effective against many diseases for centuries.

METHODS

This experimental study was conducted at Nistar Medical University and Microbiology Department of Abasyn University Peshawar from Mar 15, 2020, to Jun 15, 2020. A total of 125 samples were collected from indoor patients at Nistar and Alflah Dentistry Departments, Pakistan. Samples were collected using sterile swabs under standard microbiological practices. The samples were processed within 05 hours after collection and streaked on Nutrient agar plates which were subjected to incubation for 24 hours at 37 °C. After incubation, mixed colonies were obtained on each plate. The colonies were picked and purified by repeated sub-culturing for specific bacterial growth and used differential media (i.e. Blood Agar, Mannitol Salt Agar (MSA), MacConkey Agar and Chocolate Agar) for the growth of bacteria species. Further identification of the pure colonies was made by using Gram Staining and other biochemical tests such as Oxidase, Indole, TSI, Citrate, Urease and Catalase. (Ilyas et al., 2016). Characterization on the basis of Antibiotic, Toothpaste and Plan Extract Sensitivities

Antibiotic Sensitivity: For Antibiotic Sensitivity testing, Kirby Baur disc diffusion test was performed according to the CLSI guidelines. Amoxicillin (5µg), sulfamethoxazole (25µg), oxacillin (5µg), vancomycin (30µg), moxifloxacin (5µg), amoxicillin (30µg), ampicillin (10µg), azithromycin (15µg) were selected to check the susceptibility as described by (Abdulhaq et al., 2020).

Toothpaste Sensitivity: For Toothpaste Sensitivity testing agar well diffusion method was used. Three different concentrations of each toothpaste were made at full strength (50 mg) and 1:1 dilution. The fresh cultures from the sample were taken in broth, and the turbidity was made with McFarland Standards. The zone of inhibition around the toothpaste containing wells showed the antimicrobial activity against the bacteria. The maximum zone formed by toothpaste against bacteria were considered as sensitive toothpaste while medium zone formed against bacteria were considered intermediate toothpaste and no zone of inhibition showed the resistance of bacteria to that particular toothpaste.

Plant Extract Sensitivity: For plant extract sensitivity testing well diffusion method was used. The fresh cultures from the sample were taken and tested against A. bracteosa and C. longa extracts. Leaf, Stem and Roots of A. bracteosa and entire plant of C. longa were used in crude form and diluted with Dimethyl Sulfoxide (DMSO) according to standard procedure. The zone of inhibition around the wells showed the antimicrobial activity against the bacteria. The maximum zone of inhibition formed by plant extract against bacteria was considered as sensitive, intermediate and resistant to a particular plant extract. The plant extract was used to detect the plant extract susceptibility pattern of Streptococcus mutans, Staph aureus, Streptococcus sobrinus and Lactobacillus acidophilus.

Statistical Analysis: Mean antimicrobial activities of plant extracts were calculated against each bacterium with 95% Confidence Interval. Pearson Correlation between dose and anti-bacterial activity of plant extracts was calculated with consideration of P=0.05 with 95% Confidence Interval. All the analyses were carried through GraphPad Prism (Version 5.0) software.

RESULTS

From 125 infected samples, 27 pure species were obtained. The data indicates the percentage of bacterial isolates from teeth gum of patients. Eight different types of bacteria, i.e. Streptococcus mutans, Staph aureus, Streptococcus sobrinus, Lactococcus acidophilus, Micrococcus, Streptococcus sanguinis and Aeromonas species, were identified. Streptococcus mutans was isolated in the highest number (29.62%), followed by Lactobacillus acidophilus (22.22%), Streptococcus sobrinus (18.51%) and S aureus (14.81%) respectively. While Micrococcus spp. 3.70%, Streptococcus sanguinis 3.70%, Actinomyces viscosus 3.70% and Aeromonas 3.70% were present at the lowest percentage.

Antibiotic Sensitivity Test: In this study, the identified eight species antibiogram patterns were analyzed against eight different antibiotics. Among all used antibiotic SXT (sulfamethoxazole) showed maximum effect against all tested bacterial strains. The maximum zone of inhibition was produced against Staph aurus (33mm) while the minimum zone was observed against S. sobrinus (23mm). The most effective antibiotics were MXF (Moxifloxacin), FOX (Foxicillin), and AMC (Amoxicillin).

Table 1: Antibiotic sensitivity pattern of Isolated bacterial species from Teeth Gums.

Microorganisms	FOX (mm)	SXT (mm)	OX (mm)	VA (mm)	MXF (mm)	AMC (mm)	AMP (mm)	AZM (mm)
Staph aureus	21	33	R	R	30	13	R	10
Streptococcus mutans	18	26	22	20	29	16	10	R
Streptococcus sobrinus	15	23	R	22	23	6	R	10
Lactobacillus acidophilus	21	29	19	23	28	19	18	17
Micrococcus spp.	30	31	20	21	31	20	19	20
Streptococcus sanguinis	30	25	18	22	30	18	18	14
Actinomyces viscosus	22	32	12	21	29	19	23	9
Aeromonas	32	28	18	18	31	21	22	19

TOOTHPASTE SENSITIVITY TEST:

The diluted and undiluted form of kinds of toothpaste revealed that antimicrobial activity was at full strength in undiluted form by showing the high number of antimicrobial agents and having linear reduction with the decrease of dilution. Overall, toothpaste 1 contained high amount of Fluoride and salt is diluted, and non-diluted form showed a good effect

against all tested bacterial strains. The non-diluted one showed good activity against Micrococcus spp (34mm) while the minimum zone was seen against S. aureus (21mm). The maximum activity of diluted one was observed against Micrococcus spp. And S. mutans (26mm) by Toothpaste 2 had active ingredients Calcium and Fluoride while the minimum zone was produced against Actinomyces viscosus (14mm). Overall tooth paste 3 had calcium as active ingredient was moderate in action.

Table 2: Kinds of toothpaste sensitivities against isolated bacterial species from teeth gums

Microorganisms	Toothpaste, 1		Toothpaste, 2		Toothpaste, 3	
	Diluted	Non-Diluted	Diluted	Non-Diluted	Diluted	Non-Diluted
Staph aureus	22	26	21	26	19	22
Streptococcus mutans	17	21	26	30	19	25
Streptococcus sobrinus	16	28	23	27	16	21
Lactobacillus acidophilus	15	26	22	27	20	22
Micrococcus spp.	16	34	26	29	21	26
Streptococcus sanguinis	15	24	25	27	19	21
Actinomyces viscosus	14	29	19	22	17	21
Aeromonas spp.	16	24	21	23	18	23

Plant extracts Sensitivity test: The methanolic extract of Ajuga bracteosa was used separately (leaf, root and stem) against tested bacterial strains. Leaf extract produced a maximum zone against Streptococcus mutans (22mm) while the minimum zone of inhibition was against Streptococcus sobrinus (12mm). The methanolic extract of C. longa produced a maximum zone of inhibition against Streptococcus mutans (22mm) while the minimum activity was observed against Lactobacillus acidophilus (15mm).

Table 3: Antimicrobial activity of the plant extracts against bacterial species isolated from gums.

Plant Extracts	Streptococcus mutans	Staph aureus	Streptococcus sobrinus	Lactobacillus acidophilus	Aeromonas spp.
Ajuga bracteosa leaf	20	17	12	16	15
Ajuga bracteosa root	19	15	15	13	12
Ajuga bracteosa stem	11	19	15	14	16
Curcuma longa	22	21	16	15	18

Statistical analysis for the antimicrobial activity against Plant extracts: After getting the anitimicrobial activity of plant extracts against isolated bacteria, the results were further subjected to statistical analyses. Pearson Correlation between dose and anti-bacterial activity of plant extracts with considertion of 0.05 P value and 95% confidence interval was calculated. The analysis revealed that Ajuga bracteosa leaf extract was merely significant against Strep. pyrogens (p=0.0101), while other bacteria showed no significance at all. Curcuma longa was found to be significant only against K. pneumoniae (p= 0.9627) out of seven other bacterial isolates. Whereas Curcuma longa showed no significance against any of the bacterial strains under study.

DISCUSSION

It is well studied that the invasion of many bacteria in the oral cavity may cause various bacterial infections. Therefore, it is incredibly essential to allow early diagnosis of various pathogenic potential microbes. The identified bacteria and their strength in an oral environment could be helpful to predict the accurate progression of various periodontal diseases (Srivastava et al., 2020).

The present study was conducted to identify various bacterial strains responsible for gum disease in the Pakistani population. The study was also aimed to check the antimicrobial activity of some antibiotics usually prescribed by dentists, including a few popular toothpaste brands and two plant extracts believed to be effective against certain diseases. In this study, patients of different age group having primary, mixed and permanent dentition have participated. Both genders were included in the study; however, we have found no gender-specific bacteria.

Table 4: Antimicrobial activity of the plant extracts against bacterial species isolated from gums.

Bacterial strains		Zone of inhibition in millimeter (mm) regarding dose (E= mg)				Mean activity with 95% CI in mm	Standard Deviation	Pearson Correlation b/w Dose & Activity (P= 0.05)	R Sequare r^2
		E_1 (50mg)	E_2 (100mg)	E_3 (150mg)	E_4 (200mg)				
Ajuga bracteosa leaf	Strepto. mutans	14	14.2	14.2	14.3	14.18±o.21	0.1258	P=0.0766 (Not Significant)	0.8526
	Staph aureus	11	11	11.1	11.1	11.05±0.09	0.0577	P=0.1056 (Not Significant)	0.8000
	Strepto. sobrinus	06	6.1	6.3	6.4	6.2000±6.20	0.1826	P=0.0101 (Not Significant)	0.9800
	Lactococcus acidophilus	10	10	10.1	10.1	10.05±0.092	0.0577	P=0.1056 (Not Significant)	0.8000
	Aeromonas spp.	09	9.1	9.3	9.3	9.175±0.239	0.1500	P=0.0533 (Not Significant)	0.8000
Ajuga bracteosa root	Strepto. mutans	13	13.2	13.4	13.5	13.28±0.360	0.2217	P=0.0102 (Not Significant)	0.9797
	Staph aureus	09	09	9.1	9.1	9.050±0.092	0.0577	P=0.1056 (Not Significant)	0.8000
	Strepto. sobrinus	09	9.1	9.1	9.2	9.100±0.130	0.0816	P=0.0513 (Not Significant)	0.9000
	Lactococcus acidophilus	07	07	07	7.1	7.025±0.080	0.0500	P=0.2254 (Not Significant)	0.6000
	Aeromonas spp.	06	06	6.1	6.1	6.050±0.092	0.0577	P=0.1056 (Not Significant)	0.8000
Ajuga bracteosa stem	Strepto. mutans	05	05	05	5.1	5.025±0.080	0.0500	P=0.2254 (Not Significant)	0.6000
	Staph aureus	13	13.1	13.2	13.2	13.10±0.130	0.0816	P=0.0561 (Not Significant)	0.8909
	Strepto. sobrinus	08	9.1	9.1	9.1	9.075±0.080	0.0500	P=0.2254 (Not Significant)	0.6000
	Lactococcus acidophilus	09	08	8.1	8.1	8.025±0.080	0.0500	P=0.1056 (Not Significant)	0.8000
	Aeromonas spp.	10	10.1	10.2	10.2	10.13±0.157	0.0957	P=0.0561 (Not Significant)	0.8909
Curcuma longa	Strepto. mutans	16	16.2	16.3	16.6	16.28±0.400	0.2500	P=0.9627 (Significant)	0.9627
	Staph aureus	15	15.1	15.1	15.3	15.13±0.210	0.1258	P=0.8526 (Not Significant)	0.8526
	Strepto. sobrinus	10	10	10	10.1	10.03±0.085	0.0500	P=0.06000 (Not Significant)	0.6000
	Lactococcus acidophilus	09	09	9.1	09	9.025±0.080	0.500	P=0.06667 (Not Significant)	0.6667
	Aeromonas spp.	12	12.1	12.2	12.2	12.13±0.160	0.0957	P=0.8909 (Not Significant)	0.8909

In our study S. aureus was the predominant isolated pathogen, this result agreed with the finding conducted in Yemen by Hassan A et al., (2018) who found the dominancy of this pathogen with a percentage of 43.1%. The high frequency of S. aureus in mouth infections can be explained by the fact found by Dinges MM et al., (2000) that S. aureus often colonizes the mucous membrane of the nose, where it can cause endogenous oral cavity infections.

The study observed that all the selected antibiotic show action against the isolates expect Oxacllin (no activity against K. pneumonia, S. pyogenes and vancomycin (no activity against K. pneumonia). Our finding does not match with Okopi et al. (2015) finding that dental caries pathogens showed resistance to ciprofloxacin, ampicillin, erythromycin and amoxicillin. This report also does not agree with the reports of Sweeney et al., (2004) that resistance of oral flora to antibiotics is an international problem and may be due to excessive abuse of antibiotics. Maripandi et al. (2011) also observed the resistance pattern and found that oral pathogen resistant to penicillin, bacitracin, streptomycin, vancomycin and

chloramphenicol. Boyanova et al. (2004) found that 21% of gram-positive anaerobic cocci from dental plaque were resistant to penicillin, and 16% of them were resistant to clindamycin. Brook (2007) detected susceptibility of Veillonella species (from dental plaque) to beta-lactam but resistance to tetracycline and erythromycin while Campylobacter gracilis showed 100% sensitivity to erythromycin, but 50% to clindamycin, cephalothin and pipracillin. This study also reported the efficiency of antibiotics against oral pathogens and found that among the selected eight antibiotics used the most effective zones of inhibition were formed by sulfamethoxazole (25µg) against all pathogenic bacteria so it can be a choice of antibiotic to treat dental caries.

This study reported that undiluted toothpaste concentration gave the highest zone of inhibition and these inhibition zones tend to decrease down the concentration gradient. Other researchers found the same result that apart from the addition of antimicrobial agents in a toothpaste, the agent concentration is also paramount to achieve the most wanted outcome of reducing oral microbes (Sadeghi and Assar, 2009; Tatikonda et al, 2014; Odeleye, F. O et al., 2018).

The use of plants and plant derivatives which are known to possess preventive and therapeutic effects could contribute to oral health (Subramaniam P. et al., 2012).

This study found that extract of Ajuga bracteosa produced maximum zone against K. pneumonia while the minimum zone of inhibition was against S. pyogenes while extract of C. longa produced a maximum zone of inhibition against K. pneumonia. In contrast, the minimum activity was observed against P. vulgaris. Shah at el., conducted a study in 2012 to investigate medicinal plant extract activity on bacterial pathogens and found that the ethanol extract of A. bracteosa showed no significant activity against S. epidermidis, moderate against E. coli, while no activity was shown against other bacterial species in his study. In contrast, ethanol extract of C. longa showed significant activity against S. epidermidis and B. Subtilis, low for S. aureus, K. pneumonia and no activity against E. coli (Shah, Z., at al., 2012).

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AZ: Study Concept, design, Manuscript writing
AJ: Manuscript writing, Critical revision
AN: Concept, design, data collection, Manuscript writing
MAFZ: Manuscript drafting, Data analysis
ZN: Concept, design, data analysis
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AA: Critical revision